STANDARD OPERATING PROCEDURE

BLOOD ALCOHOL ANALYSIS (METHOD 2)
(Revised 12/13/06)

Principle:

Headspace chromatography is based on Henry's Law which states, for dilute solution, the solubility of a gas in a liquid expressed as a mole fraction depends upon the pressure of the gas. There is a fixed ratio between the mole fraction of the gas in the air and the mole fraction in the liquid. This ratio remains constant for a given temperature.

Specimens:

Optimum sample volume-3 ml or greater.

Samples which contain less than 3 ml may be analyzed.

Acceptable specimens are whole blood, urine, biological fluids or other alcoholic solutions. Other specimens may be analyzed with the approval of the laboratory director or designee.

Blood: The preferred sample is blood that is submitted in the ND Crime Laboratory BAC Kit. These kits have sterile Vacutainer tubes containing sodium fluoride and potassium oxalate.

Urine: The preferred sample is urine that is submitted in the ND Crime Laboratory Urine Kit.

Refrigerators should be used for specimen storage.

Equipment:

1. Gas Chromatograph (GC) – Varian 3400CX , Varian 3400
   1) FID detector or equivalent for volatiles
   2) Column temp. = 40°C
   3) Restek RTX-BAC1, Restek RTX-BAC2 or equivalent column
   4) Gas flows: H2 carrier 14 mL/min
      H2 FID 17 mL/min
      N2 FID 28 mL/min
      Air FID 300 mL/min
      Split 15mL/min
      Septum Purge 5 mL/min
   5) Injector Temp. = 200°C
   6) Detector Temp. = 200°C

2. Headspace autosampler
   a. CombiPal
      i. Cycle HS-INJ
      ii. Syringe 2.5mL-HS
      iii. Sample Volume 1000 uL
      iv. Incubat Temp 60.0° C

v. Incubat Time 0:11:50
vi. Agi Speed 250 rpm
vii. Agi On Time 5 s Agi Off Time 55 s
viii. Syringe Temp 75° C
ix. Fill Speed 100uL/s
x. Inject to GCInj 1
xi. Inject Speed 250 uL/s
xii. Pre Inj Del 500 ms Pst Inj Del 500 ms
xiii. Syr Flushing 0:01:30
xiv. GC-Runtime 0:03:30

b. Tekmar 7000
i. Platen Temp: 65° C Platen Equil: 0.50 min
ii. Sample Equil: 15 min
iii. Vial Size: 22 mL
iv. Mixer: On Mix: 0.5 min Mix Power: 1
v. Stabilize: 0.50 min
vi. Pressurization: 0.50 min Press. Equil: 0.25 min
vii. Loop Fill: 0.15 min Loop Equil: 0.05 min
viii. Inject: 0.50 min
ix. Sample Loop: 120° C Line Temp: 120° C
x. Transfer Line Back Pressure: 12.5 psi
xi. Vial Pressurization: 12.0 psi
xii. Injection / vial: 1
xiii. GC Cycle Time: 4

NOTE: Temperatures, pressures and other parameters in Equipment 1 and 2 are suggested operating conditions and may need to be altered to obtain optimum chromatographic results.

3. Atlas chromatography data system, chromatography software or integrator.

4. Vials, caps, septa, and crimper.

5. Other laboratory supplies
1) pipettes (SMI and/or equivalent)
2) automatic delivery pipette or Repipet
3) weighing bottles and lids
4) volumetric flasks and stoppers (various sizes)
5) analytical balance
6) polyethylene bottles (500 mL)
7) storage vials with caps
8) beakers (various sizes)
9) pipettes

NOTE: As determined by analysts; appropriate lab supplies, equipment or glassware may be substituted for analytical procedure.

Reagents:

1. n-Propanol, $CH_3CH_2CH_2OH$, analytical grade. **Flammable, may be harmful if swallowed, inhaled, or absorbed through the skin.**

2. Ethanol, CH_3CH_2OH, 200 proof, USP grade. **Flammable, may be harmful if swallowed, inhaled, or absorbed through skin.**

3. Sodium fluoride, NaF, analytical grade. **May be fatal if inhaled or swallowed.**

4. Sodium hydrosulfite, $Na_2S_2O_4$, analytical grade. **Flammable. May ignite with moisture and air. Harmful if swallowed. Causes irritation.**

5. Ammonium sulfate, $(NH_4)_2SO_4$, analytical grade.

6. Ethanol Calibrators: Made with 200 proof USP grade ethanol and reverse osmosis de-ionized water. Store in refrigerator. Expiration date is 2 months from date of preparation.

7. Diluent: Made with analytical grade ammonium sulfate, analytical grade sodium hydrosulfite and reverse osmosis de-ionized water. Store at room temperature. Expiration date is 6 months.

8. Internal Standard: Made with analytical grade n-propanol and diluent. Store at room temperature. Expiration date is 6 months.

9. Blood Bank: **Use Universal Precautions when handling biohazardous material.** Prepared by purchasing whole blood or packed Red Blood Cells (RBCs) from United Blood Services of Bismarck or equivalent vendor and adding analytical grade sodium fluoride. Prepare at a concentration of 10 mg/mL. Expiration date is 6 months. Store in refrigerator.

10. Aqueous Commercial Controls: Target concentration of 0.10 g% ethanol by weight. Concentration ranges of controls may be used between 0.02 to 0.50 g% ethanol by weight. Expiration date is determined by manufacturer. Store at room temperature.

11. Commercial ethanol standards may be purchased. Concentration range of 0.002 g% to 1.000 g%. Expiration date is determined by manufacturer. Store at either room temperature or refrigerate until opened. Once opened, store in refrigerator.

Preparing Standard Ethyl Alcohol Solutions : (See Table I)

1. Use calibrated pipettes (or equivalent).

2. Use pure anhydrous ethyl alcohol.

3. Fill the appropriate size volumetric flask to about 4/5 full with distilled water.

4. Place approximately 10-15 mL of distilled water into weighing vessel. If required fit with lid, place on analytical balance and tare.

5. Deliver the pure anhydrous alcohol to the weighing vessel and note the weight in grams to 4 decimal places.

6. Quantitatively transfer the contents of the weighing bottle into the appropriate size volumetric flask, rinsing the weighing vessel several times with distilled water.

7. Fill the volumetric flask to the mark with distilled water and mix contents thoroughly.

8. Transfer into 500 mL polyethylene bottles and store in refrigerator.

9. Check new standards against the previous standards for accuracy. Analyze 4 samples of each by GC. Standards <0.10 g% must be within ± .005 g% of weighed value, while standards > 0.10 g% must be within 5% of weighed value.

TABLE I		
Target Alcohol Concentration (g%)	Volumetric Flask Size	Standard Solution Concentration
0.015	1 L	0.015 g% ± .005 g%
0.050	0.5 L	0.050 g% ± .005 g%
0.150	0.5 L	0.150 g% ± 5%
0.350	0.5 L	0.350 g% ± 5%
0.550	0.5 L	0.550 g% ± 5%

These solutions will be used in preparing the calibration curve.

10. Commercially prepared ethanol standards may also be used for the preparation of the calibration curve.

Preparation of Diluent and Internal Standard Solutions:

1. The diluent solution is prepared by dissolving 132 grams of ammonium sulfate and 17.4 grams of sodium hydrosulfite per liter of reverse osmosis distilled (ROD) water.

2. The internal standard (IS) solution is prepared by diluting a weighed volume (250 uL) of n-propanol per liter of diluent solution to obtain a concentration within the range of 0.018 g% to 0.022 g%.

Preparation of Volatiles Solution

1. The "volatiles" solution is a dilution of 25-50 uL each of methanol, acetone, ethanol, iso-propanol and n-Propanol into a 100 mL volumetric flask.

2. The flask is approximately half filled with distilled water before the addition of the various volatiles and then filled to the mark with distilled water.

3. Invert several times to mix contents.

4. This solution is for qualitative use only.

5. Store in refrigerator.

Preparation of Standards, Controls, Case Samples, Blank, and Zero for Analysis

1. Table II summarizes the preparation of each required item for analysis. Table III shows the procedure for preparing the calibrators.

TABLE II					
	Volume Used	Amt of Blood Added	Amt of ROD Water Added	Amt of Diluent Solution	Amt of IS Solution
Standards	100 uL	100 uL	---	---	2 mL
Commercial Control(s)	100 uL	100 uL	---	---	2 mL
Blank	---	100 uL	100 uL	2 mL	---
Zero	---	100 uL	100 uL	---	2 mL
Volatiles	100 uL	100 uL	---	---	2 mL
Sample - Blood	--	100 uL	100 uL	--	2 mL
Sample – Urine or Aqueous Sample	100 uL	--	100 uL	--	2 mL

2. Each standard ethyl alcohol solution is prepared in singlet. Blank, zero, and volatiles are prepared in singlet.

3. Commercial controls are prepared as needed. Case samples are prepared in duplicate. Samples and controls may be analyzed more than once.

4. Once all components are placed in the labeled vial, it is capped and crimped.

TABLE III - Standard Curve Preparation				
Standard Target Concentration	Amt. Std. Added	RO Dist Water	Blank Blood	IS Soln
0.015 g%	100 ul	0	100 uL	2 mL
0.050 g%	100 ul	0	100 uL	2 mL
0.150 g%	100 ul	0	100 uL	2 mL
0.350 g%	100 uL	0	100 uL	2 mL
0.550 g%	100 uL	0	100 uL	2 mL

Sample Loading Procedure

1. An autosampler worksheet is prepared indicating the position of each vial in the carousel or tray of the auto-sampler. As samples are added to the carousel/tray, their case number is entered into the autosampler worksheet. A carousel/tray may be reloaded as needed due to sample number.

2. Upon completion of the analysis, the position and identity of the vials should be compared to the worksheet to verify the injection sequence prior to removal of the vials from the autosampler carousel/tray.

3. The number of controls analyzed should not be less than 25% of the case samples being tested.

Sample Analysis

1. The proper sequence for beginning an alcohol analysis would be to run the 5 ethyl alcohol standards, then the blank, zero, and volatiles solutions, followed thereafter by a constant pattern of a control, then case sample (in duplicate) and ending with a control.

2. The standard curve should be analyzed by linear regression analysis. The correlation coefficient of the line will be calculated. If the correlation coefficient is not greater than or equal to 0.999, then the standard curve should be rerun.

3. As long as the concentrations of controls continue to be in the acceptable range, there is no need to rerun the standard curve. However, if any control falls out of acceptable range then the case sample prior to and immediately following that control must be reanalyzed.

4. If the concentration sample is greater that the highest standard concentration, the sample may be diluted with ROD water and then re-analyzed. The reported value will then be adjusted according to the amount of the dilution.

Acceptance Criteria

1. The correlation coefficient as determined via linear regression analysis of the five standards must be ≥ 0.999.
2. The reported concentration of all controls > 0.10 g% must be within 5 % of the expected value. Controls < 0.10 g% should be within 0.005g% of the expected value. If a control falls out of range, the sample on either side of the control should be repeated along with a control.
3. In the analysis of a case sample, the deviation between that average and either one of the two concentration values should not be greater than 3 %. If the difference is greater than 3 % then the case sample must be retested.

Sample reporting

1. The case results should be generated as Form 101.
2. The Analytical Report (Form 107 or 107-U) should be completed by the analyst.
3. If the size of the sample submitted is less than what is necessary to perform the analysis in duplicate, the Analytical Report (Form 107 or 107-U) should reflect that the quantity is insufficient for analysis.
4. A certified copy of the Submission Form (Form 104 or 104-U) and Analytical Report (Form 107 or 107-U) should be prepared and sent to the submitting agency.
5. The result should be recorded on the Case Jacket Review, if applicable.
6. A Peer Review of the analysis will be performed before the reporting of the results.

Headspace Analysis Files and Chromatograms

1. A headspace file will be generated to include:
    a. Standards, zero, blank and volatiles chromatograms,
    b. Alcohol Analysis Worksheets
    c. Computer generated spreadsheet or chromatography software: information, QC summary, and sample summary
2. At a minimum, the file will be labeled as headspace analysis, date and analysts initials.
3. The headspace files will be filed chronologically.
4. Chromatograms and raw data are stored electronically on a computer server. Chromatograms and data will be printed out on an as needed basis.
5. Data will be analyzed and reported by either computer spreadsheet or chromatography software.

Sample Storage

1. After reporting the results to the submitting agencies the sample should be placed in appropriate storage and the sample number will be marked on a Blood Tube Storage worksheet.

Manufacturers and vendors for chemicals, reagents, and supplies include, but are not limited to:

Aaper Alcohol
Nerl Diagnostics
Fisher Scientific